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How temperature stress changes carrot flavor: Elucidating the genetic determinants of undesired taste in carrots

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Project award year: 2014
Three year research project

Abstract

Global climate change and warming temperatures represent the greatest future challenge for global food production and quality. In this project, we will define the genetic factors of climate-associated taste deficiencies in carrot. Carrot is considered one of the leading horticultural crops in the world in terms of its nutritional value, health benefits, and unique flavor based on its high content of carotenoids and volatile aroma compounds. In recent years, carrot genotypes of different color with improved nutraceutical attributes have been developed. When exposed to high growth temperatures, carrots develop an undesired harsh and bitter taste caused by the accumulation of terpene metabolites. This taste deficiency represents a quality defect to carrot breeders and large-scale growers and needs to be minimized for successful marketing of carrot crops. Surprisingly, the genetic determinants of bitter and harsh flavor in carrot and their response to temperature stress are not well characterized. We started to elucidate these factors in different carrot cultivars by investigating the biosynthesis of volatile terpenes, which represent the predominant flavor compounds in carrots. Also, up to date we identified and characterized two terpene synthase enzymes, one of which produces (*E*)-β-caryophyllene, a major terpene component in carrot root. Both TPSs may contribute to the observed variation in volatile terpene formation.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Abstract - Poster	0	5	0
Abstract - Presentation	0	1	0
Other	0	2	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Ph.D. Student	Muchlinski	Andrew	Virginia Tech	USA

Description of the cooperation:

Cooperation has been extremely close during the first year of the project. Initially, the collaborating partners communicated by email and skype to discuss the experimental approach and coordinate the project. Carrot seeds, RNA, cDNA, plasmid DNA and constructs were exchanged between both countries.

Evaluation of the research achievements as related to the original research proposal and objectives:

1. We have completed the analysis of volatile terpene profiles and transcriptomes of four different colored carrot cultivars
2. We have performed a comprehensive analysis of the entire carrot *TPS* gene family based on the genome and trancriptome of the doubled-haploid orange carrot DH1. A total of 21 *TPS* genes were cloned and recombinant proteins functionally characterized. Several of these genes encode enzymes whose products are predominant components of the carrot DH1 root terpene profile.
3. To determine the change of terpene metabolism in 4 colored cultivars under elevated temperature conditions, we analyzed terpene profiles of these cultivars grown under three different temperature regimes. In correlation with these results, changes of transcript levels of selected *TPS* genes were determined.
4. We attempted to generate transgenic RNAi plants to knock down the gene *DcTPS1* and *DcTPS2,* of which *DcTPS1* is responsible for the synthesis of the predominant carrot sesquiterpene compound (*E*)-β-caryophyllene. Unfortunately, no stable RNAi lines could be established for both of these genes due to possible toxic off-target effects.

Achievements

Effects of elevated growth temperatures on changes in terpene profiles of different colored carrot cultivars by targeted terpene metabolite profiling (Ibdah)

To determine changes in volatile terpene profiles at three basic diurnal temperatures (16, 22, and 28 ºC), colored carrot cultivars from the **Simon** lab were used (see **Table 1).** This germplasm was selected for all subsequent experiments since it represents established genetic resources for consistent and comparative metabolite and gene expression analyses.

Table 1. Carrot genetic resources for terpene metabolite and transcriptome analysis.

Inbred	Seed source	Root color
B493B	920-1	Orange
P7262	931-1	Purple orange
R6637	370-1	Red
Y9244A	939-2	Yellow

Seeds were first germinated and seedlings established in the green house for four weeks at a 20/16ºC (±1°C) day/night temperature regime under natural daylight conditions. The seedlings were then transferred into containers and placed into phytotrons, and a diurnal temperature of 16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night was applied. We harvested root samples (triplicate) of the different carrot cultivars after 11 weeks and stored the tissue at -80ºC for subsequent terpene volatile analysis and transcriptome and quantitative reverse transcriptase (qRT)-PCR profiling.

For terpene analysis, replicate samples of fresh root carrots (1 g) of each cultivar were ground to a uniform powder and incubated for 2 h in 20% NaCl containing an internal standard. Volatile compounds were analyzed by automated SPME (solid phase microextraction)-GC-MS. Individual compounds were quantified by SPME upon thorough calibration with authentic standards.

The examination of the volatile compound composition of freshly harvested root tissues from different colored carrot cultivars growing in our experimental station at different growth temperatures (16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night), indicated that volatile compounds in these organs consist mainly of mono- and sesquiterpene hydrocarbons (**Supplementary S Table 1**). Some fatty acid-derived volatiles such as *n*-undecane and tridecane were also observed (**Supplementary S Table 1**).

There were significant differences in volatile composition between different carrot cultivars and growth conditions. For example, the accumulation value of several monoterpenes such as *α*-pinene, *β*-myrcene, and limonene were increased by increasing the growth temperatures (Table 2 and Fig. 1). It was also noteworthy that the roots of the different carrot cultivars showed increased levels of *γ*-cadinene, and *δ*-cadinene (Table 2 and Fig. 2); z-*γ*-bisabolene (Table 2 and Fig. 3), by increasing the growth temperatures.

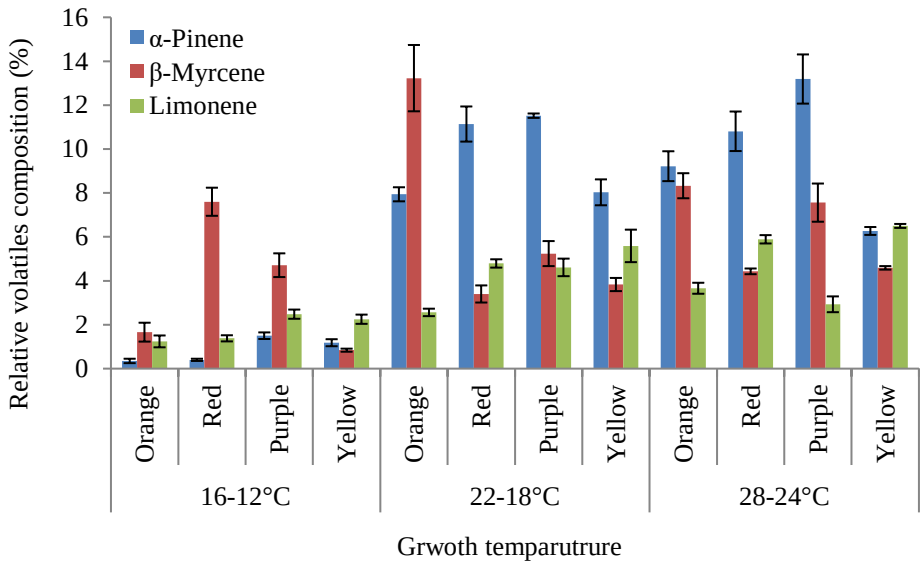

Figure 1: Effect of the temperature on selected monoterpenes accumulation. The different carrot cultivars were growing in our experimental station at different growth temperatures (16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night). All analyses were performed using five biological replicates.

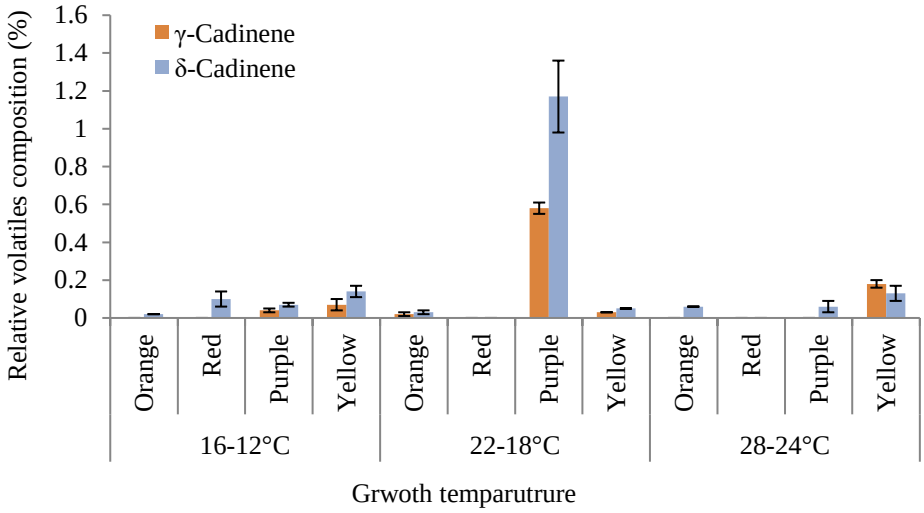

Figure 2: Effect of the temperature on accumulation of γ-cadinene and δ-cadinene. The different carrot cultivars were growing in our experimental station at different growth temperatures (16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night). All analyses were performed using five biological replicates.

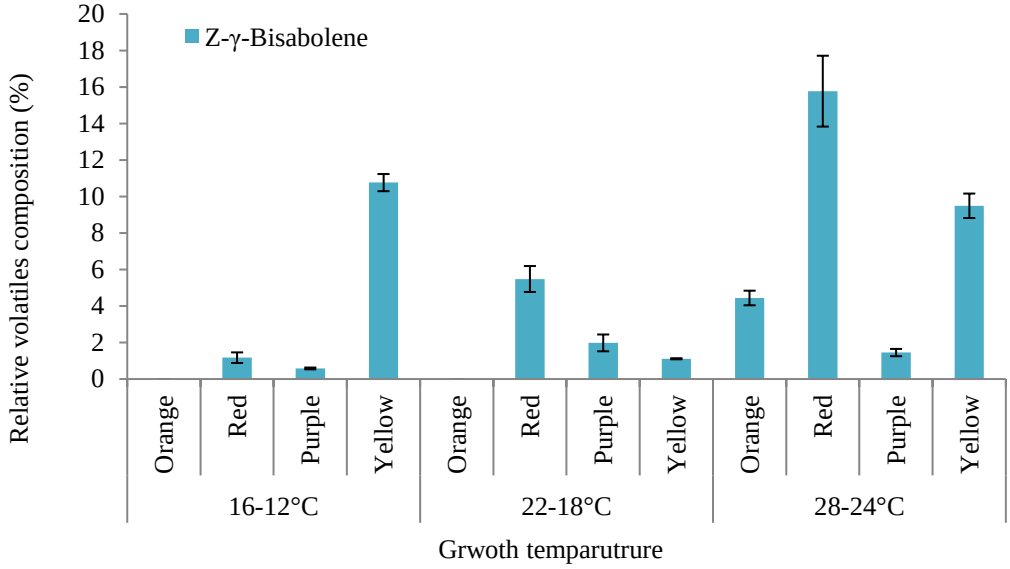

Figure 3: Effect of the temperature on Z-γ-bisabolene accumulation. The different carrot cultivars were growing in our experimental station at different growth temperatures (16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night). All analyses were performed using five biological replicates.

Establishing Genome and Transcriptome Resources for TPS Gene Identification and Expression Profiling (Tholl, Jensen, Simon)

In an initial attempt to functionally characterize root-specific members of the carrot *TPS* gene family, full length *TPS* gene transcripts were detected by de novo assembly of an already existing transcriptome data set of the purple cultivar B7267 (Table 3) and an orange cultivar (B6274) [Iorizzo et al., 2011, BMC Genomics. 12:389. De novo assembly and characterization of the carrot transcriptome reveals novel genes, new markers, and genetic diversity]. The **Jensen** group identified at least 6 full length *TPS* cDNAs, of which three were predicted to be monoterpene synthases and three were predicted to be sesquiterpene synthases (Table 3).

Table 3. Detection of carrot *TPS* genes based on *de novo* assembly of Illumina sequences of two carrot genotypes (Iorizzo et al., 2011). Six contigs correspond to full length *TPS*.

Carrot EST Contig	Number of mapped reads per *TPS* Contig		Predicted TPS function according to best Blast Match (Genbank) >50% AA
	Cultivar B6274 (orange)	Cultivar B7267 (purple)	
1324	1079	2160	Monoterpene synthase
21245	2392	6925	Monoterpene synthase
43814	3119	940	Monoterpene synthase
4929	5726	304	Sesquiterpene synthase
52846	1045	3478	Sesquiterpene synthase
58617	602	598	Sesquiterpene synthase
	Total RNA-Seq reads per cultivar: 28,363,561	Total RNA-Seq reads per cultivar: 33,029,462	

Identification and Functional Characterization of Carrot *TPS* Genes (Ibdah)

In addition to the previous functional characterization of *DcTPS1* and *DcTPS2*, [Yahyaa et al., 2015; J Agric Food Chem. 63:4870-8. Identification and Characterization of Terpene Synthases Potentially Involved in the Formation of Volatile Terpenes in Carrot (*Daucus carota* L.) Roots.], the **Ibdah** lab isolated RNA from orange colored carrot roots (B493B) using the Spectrum Plant Total RNA Kit (Sigma-Aldrich), and performed an RT-PCR to yielded a 1764 bp fragment for DcTPS1324, and a 1701 bp fragment for DcTPS58617 (Table 3), respectively. cDNAs were

ligated into the pEXP5-CT/TOPO TA expression vector (Invitrogen Corporation, Carlsbad, CA), to produce pEXP-DcTPS-1324, pEXP-DcTPS-58617, respectively, in which the DcTPS-1324, and DcTPS-58617 coding sequences were fused with a His-tag-coding extension at the C terminus. The constructs were verified by sequencing and transformed into *Escherichia coli* TOP10 cells.

Following heterologous expression of DcTPS-1324, and DcTPS-58617 as recombinant enzymes and purification by affinity Ni-agarose chromatography, enzyme activity assays were performed using GPP as substrate.

Recombinant DcTPS1324 converted GPP to terpinolene, as the primary product, along with limonene, *α*-phellandrene, *β*-myrcene, and *α*-pinene (Fig. 4).

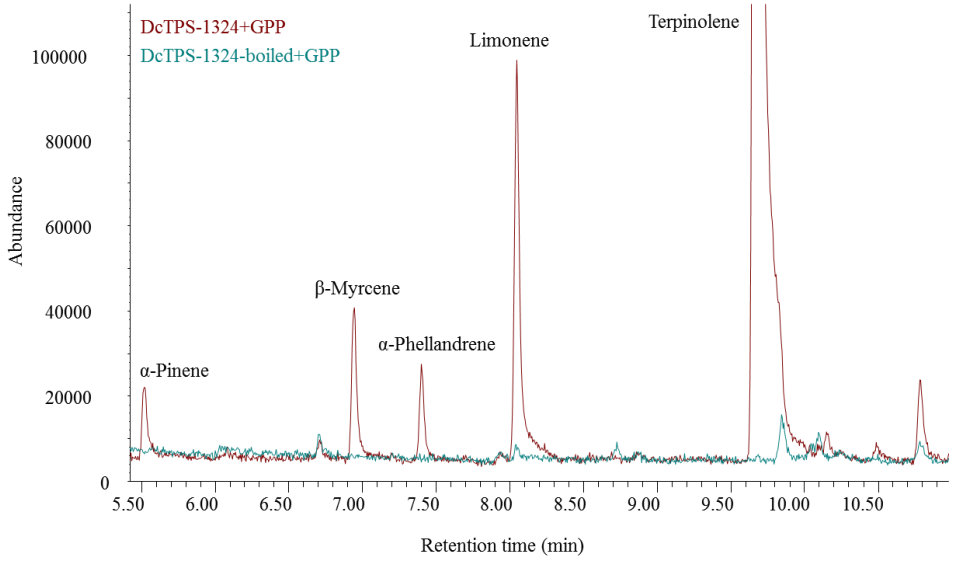

Figure 4: GC-MS analysis of the products generated in vitro by Ni-NTA-purified recombinant DcTPS-1324 protein with GPP as a substrate. Enzymatic products were analyzed by auto-HS-SPME-GC-MS (brown traces). Control with boiled DcTPS-1324 (blue traces). Identification of the products was performed by comparison of the mass spectra with those of authentic standards and according to the retention time and by mass spectral library comparison.

GC-MS analysis of the reaction products catalyzed by DcTPS-58617 with FPP as a substrate (Fig. 2) identified at least 32 sesquiterpenes, with *δ*-cadinene and germacrene D as two major products (peaks #24 and #17, respectively, in Fig. 5).

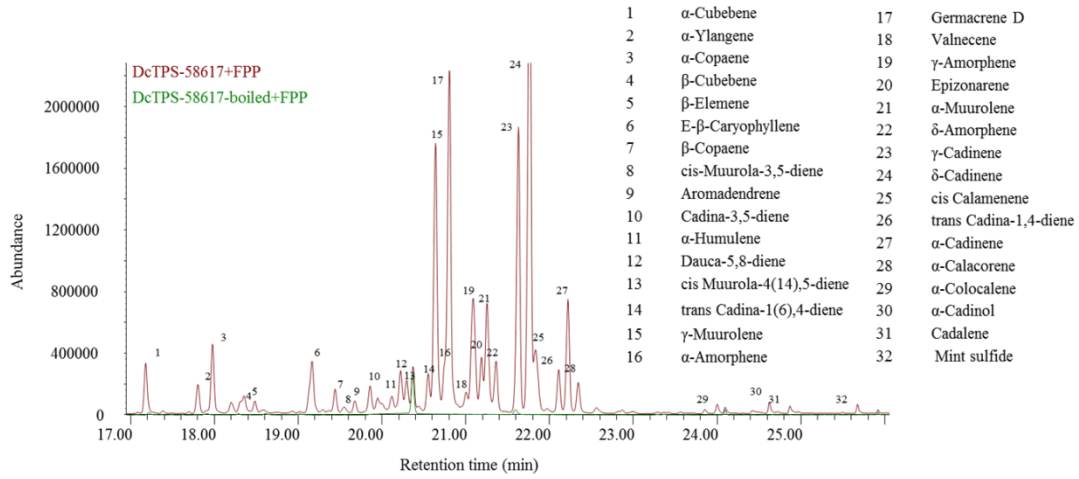

Figure 5: GC-MS analysis of the products generated in vitro by Ni-NTA-purified recombinant DcTPS-58617 protein with FPP as a substrate. Enzymatic products were analyzed by auto-HS-SPME-GC-MS (brown traces). (B) Control of boiled DcTPS-58617 (green traces). Identification of the products was performed by mass spectral library comparison.

Identification and Functional Characterization of Carrot *TPS* Genes (Tholl, Simon)

To identify additional functionally active TPSs with expression in carrot roots, the **Tholl and Simon labs** mined the published carrot genome and transcriptome of the doubled-haploid orange carrot DH1 [Iorizzo et al., 2016, A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution, Nat Genet. 48:657-66]. Since the last report, the screening of full length genes has been refined.

This screening resulted in the detection of 43 full length *DcTPS* sequences, which include the sequences shown in Table 3. Amino acid alignment and phylogenetic analysis of the 43 full-length TPS genes indicated that carrot TPSs can be organized into 6 TPS sub-families according to the classification by Chen et al. (2011) [The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant J, 66:212-229], see Figure 6.

Based on RT-PCR amplification of full length transcripts and quantitative RT-PCR analysis, 16 genes (excluding the genes already described by Ibdah) with expression in roots (DcTPS03, DcTPS10, DcTPS11 DcTPS14, DcTPS15, DcTPS25, DcTPS26, DcTPS28 and DcTPS30) and high expression in leaves or the petiole (DcTPS04, DcTPS07, DcTPS19, DcTPS23, DcTPS42, DcTPS48, DcTPS53) were amplified as full length cDNA clones and expressed in *E.coli* using the pET28 prokaryotic protein expression vector. Truncated proteins were expressed in the case of predicted plastidial transit peptides for monoterpene synthases or diterpene synthases.

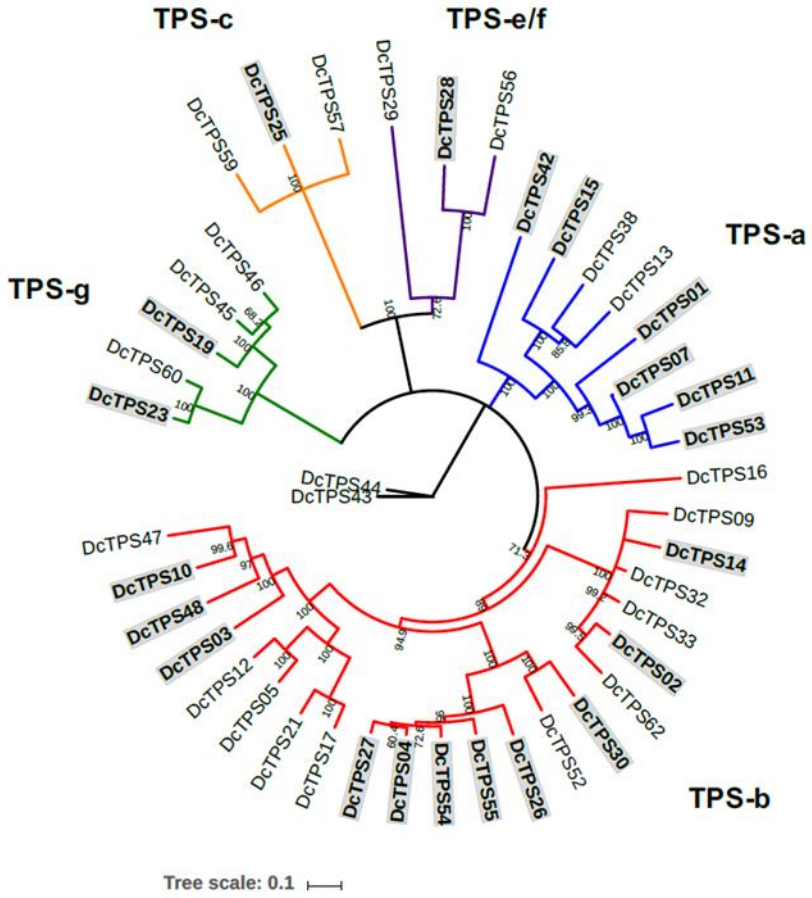

Figure 6. Neighbor-joining consensus tree (Geneious v8.0.2, Kearse et al. 2012, *Bioinformatics*) predicted from the MUSCLE (Edgar 2004, *Nucleic Acid Res.*) alignment of 43 full-length terpene synthases identified from carrot (cv. DH1). Enzymes analyzed in this study and by Yahyaa et al. 2015 are highlighted in bold. Predicted transit peptides were truncated prior to alignment based on ChloroP predictions (Emanuelsson et al. 1999, *Protein Sci.*), and TPS sub-family alignments with published TPSs (see Supplemental). Bootstrap replicates = 1000. Additional phylogeny formatting was done using the Interactive Tree of Life (iTOL v1.0, Letuniuc and Bork 2006, *Bioinformatics*).

The Tholl lab performed in vitro TPS assays with the recombinant partially purified TPS proteins using all possible TPS substrates (GPP-geranyl diphosphate, NPP – neryl diphosphate, (*E*,*E*)-FPP-all-*trans*-farnesyl diphosphate, (*Z*,*Z*)-FPP-all-*cis*-farnesyl diphosphate, GGPP-geranylgeranyl diphosphate). All substrates were provided at a concentration of 60 µM and products were analyzed by headspace SPME-GC/MS during 5 min of incubation at 30°C. Gas chromatograms of terpene products are shown in Figure 7. A summary of all characterized DcTPS proteins is shown in Table 4.

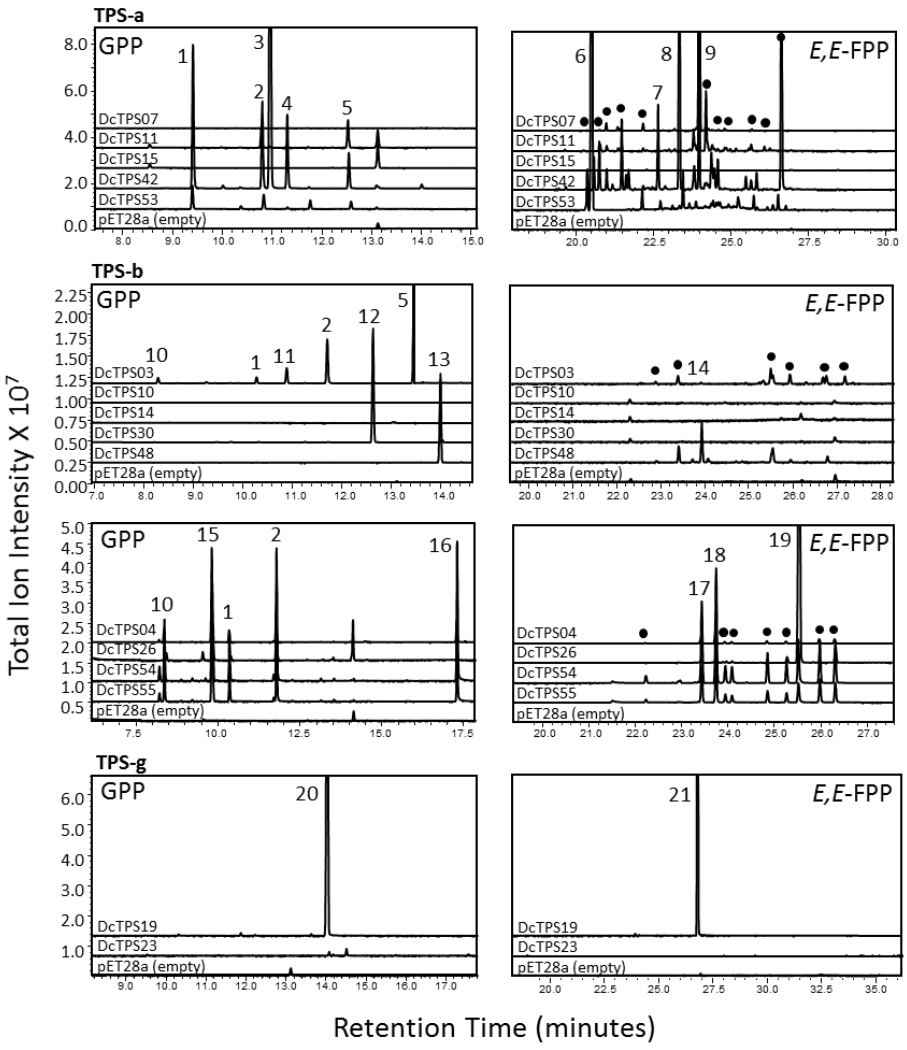

Figure 7. SPME-GC-MS analysis of the products formed in vitro by partially purified monoterpene and sesquiterpene synthase enzymes. Assays were incubated for 5 minutes in the presence of a SPME fiber prior to thermal desorption as described in "Material and Methods". 1: β-myrcene, 2: limonene, 3: *Z*-β-ocimene, 4: *E*-β-ocimene, 5: α-terpinolene, 6: Δ-elemene, 7: α-patchoulene, 8: β-patchoulene, 9: germacrene-D, 10: α-pinene, 11: α-phellandrene, 12: γ-terpinene, 13: linalool, 14: β-farnesene, 15: sabinene, 16: α-terpineol, 17: bergamotene, 18: sesquisabinene, 19: β-bisabolene, 20: linalool, 21: nerolidol. Compound identification is based on comparisons to authentic standards and essential oils provided by Dr. Tobias Köllner (MPI-CE). GPP: geranyl diphosphate, *E*,*E*-FPP: all-*trans*-farnesyl diphosphate.

Recently, Keilwagen et al. (2018) performed an extended analysis of the DH1 genome, which led to the identification of 22 additional predicted *TPS* gene models. However, a large proportion of these predicted genes is not expressed in roots or only a low levels. Predictions of QTLs for the synthesis of particular terpene compounds prompted us to functionally characterize 3 additional *TPS* genes as part of a predicted QTL for "sabinene" synthesis. We were able to correlate this QTL with four sabinene synthases (DcTPS04, 26, 54, 55). However, other predicted QTLs could not be confirmed based on by our *TPS* gene characterization.

Table 4: Functionally characterized carrot TPSs. TPS product identification is based on comparisons with mass spectral libraries and authentic standards.

		Predominant enzymatic products from different substrates				
DcTPS	Subfamily	GPP	NPP	(*E*,*E*)-FFP	(Z,Z)-FPP	GGPP
1 (s)	a	none	n.d.	(*E*)-*β*-caryophyllene	n.d.	n.d.
2 (m)	b	β-myrcene geraniol	n.d.	n.d.	n.d.	n.d.
3 (m) 1324 (Table 3)	b	α-terpinolene	n.d.	n.d.	n.d.	n.d.
4 (m)	b	sabinene limonene	sabinene limonene	α and β-bisabolene isomers	bergamotene sesquisabinene *β*-bisabolene	n.d.
7 (s) 58617 (Table 3)	a	n.d.	n.d.	multi-sesquiterpene (germacrene d, *δ*-cadinene)	n.d.	n.d.
10 (m)	b	multi-monoterpene	multi-monoterpene	farnesene, bisabolene isomers (low activity)	α-bisabolene isomers	n.d.
11 (s)	a	limonene α-terpinolene	limonene α-terpinolene	germacrene D bicyclogermacrene	γ-bisabolene isomers	cembrene isomer
14 (s)	b	Limited activity	Limited activity	Limited activity	Limited activity	Limited activity
15 (s)	a	Limited activity	Limited activity	Limited activity	Limited activity	Limited activity
19 (m)	g	linalool	linalool	Nerolidol	nerolidol	
23 (s)	g	Limited activity	Limited activity	Limited activity	Limited activity	Limited activity
25 (d)	c	n.d.	n.d.	n.d.	n.d.	*ent*-CPP
26 (s)	b	sabinene limonene	sabinene limonene	α and β-bisabolene isomers	bergamotene sesquisabinene β-bisabolene	unidentified diterpene hydrocarbon
27 (m)	b	Not functional	Not functional	Not functional	Not functional	Not functional
28 (d)	e	n.d.	monoterpene traces	β-fanesene isomer	β-fanesene isomer	*ent*-kaurene
30 (m)	b	γ-terpinene	γ-terpinene	n.d.	n.d.	n.d.
42 (s)	a	β-myrcene β-ocimene	limonene α-terpinolene	multi-sesquiterpene (pathchoulene)	α and y-bisabolene	n.d.

					isomers	
48 (m)	b	linalool	linalool	none	none	n.d.
53 (s)	a	β-myrcene	β-myrcene	Δ-elemene	Δ-elemene	n.d.
54 (m)	b	sabinene limonene	sabinene limonene	α and β-bisabolene isomers	bergamotene sesquisabinene β-bisabolene	n.d.
55 (m)	b	sabinene limonene	sabinene limonene	α and β-bisabolene isomers	bergamotene sesquisabinene β-bisabolene	n.d.

At least two of the characterized monoterpene synthases (DcTPS30 and DcTPS03) appear to produce the major monoterpenes γ-terpinene and α-terpinolene among other monoterpenes that have been detected in the root of DH1 and other carrot cultivars, while DcTPS1 makes the predominant sesquiterpene (*E*)-*β*-caryophyllene and several of the other TPSs are capable to produce major carrot sesquiterpene products such as bisabolenes, germacrene D, and cadinenes from all-*trans* or all-*cis* FPP (Figure 7).

Based on the obtained results by the **Ibdah and Tholl labs**, a comprehensive manuscript describing the carrot *TPS* gene family and changes of terpene profiles and gene expression at elevated temperature will be submitted in August/September 2018.

RNAi-mediated silencing of TPS genes (Simon)

Given the availability of full *TPS* gene sequence and terpene metabolite profiling in year 1, the development of RNAi constructs and carrot transformants to evaluate RNA-mediated silencing was initiated in year 1. This research revealed that DcTPS1 from carrot EST contig 4929 is a true sesquiterpene synthase, which mainly produces (*E*)-*β*-caryophyllene, the predominant sesquiterpene in carrot roots. Furthermore, DcTPS2 from contig 43814 was found to be active in geraniol biosynthesis. These two genes were chosen for elucidation the role of *TPS* genes by applying RNAi methodology.

Sterile seedlings of B493 dark orange carrots are being used throughout this part of the project and in year 3 parallel efforts were initiated using inbred B2566. To develop RNAi constructs, the 251 nt unique sequence derived from DcTPS1,and 220 nt of DcTPS2 were amplified and introduced into the pENTR/D-TOPO vector. A construct with no DcTPS sequence was used as a control for comparison. Constructs were subsequently inserted into the plant

transformation *Gateway*-ready binary RNAi *vector pK7GWIWG2* containing kanamycin resistance as a selectable marker with a lambda reconstruction (LR) recombination site in the sense and antisense orientation under control of the constitutive cauliflower mosaic virus (CaMV35s) promoter. Recombinant pK7GWIWG2(II)-DcTPS plasmids were selected and their accuracy confirmed by sequencing of inserts. The resulting plasmids were named pK7-TPS4259 and pK7-TPS4381.

Agrobacterium tumefaciens strain GV3101 containing plasmid pMP90 was used for transformation throughout this experiment. Electroporation was used to introduce the RNAi constructs into Agrobacterium GV3101. The presence of RNAi constructs was confirmed by colony PCR. For carrot transformation, a single colony of transformed GV3101 Agrobacterium suspension was used to inoculate sterile hypocotyls with co-cultivation for 2-3 day at 30°C in the dark. For callus induction, transformed tissue was placed on selective MS medium containing 250 μg/mL cefotaxime and 200 μg/mL kanamycin, and incubated at room temperature (22°C) under cool white fluorescent light.

Somatic embryos began to appear between 6-8 weeks for B493, and between 5-7 weeks for B2566, and growth of transformants with DcTPS1 and DcTPS2 in both B493 and B2566 was slower than typical cultures, or transformants with no DcTPS sequence. Transformants were subcultured to fresh hormone-free MSI medium every 3-4 weeks to initiate rooting. Calli of transformants with DcTPS1 and DcTPS2 in both B493 and B2566 became green on upper surfaces and demonstrated some signs of differentiation with small leaf primordia and short rootlets after 3-7 months, in contrast to those calli with no DcTPS sequence, or untransformed calli that demonstrated prolific leaflet and root formation and similar regeneration progress. After rooting and acclimatization, a few of the DcTPS transformants regenerated weak plantlets with 1-3 leaflets no larger than 1cm and roots of a similar length. No visible growth occurred after 6 months. The successful growth and regeneration untransformed calli and transformed calli with no DcTPS sequence indicated that the DcTP1 and DcTPS2 interfered with normal growth and development. Reports of toxic off-target effects of RNAi systems has been reported in other organisms, but were not expected in this project.

Evaluation of the research achievements as related to the original research proposal and objectives:

1. We have completed the analysis of volatile terpene profiles and transcriptomes of four different colored carrot cultivars.
2. We have performed a comprehensive analysis of the entire carrot *TPS* gene family based on the genome and trancriptome of the doubled-haploid orange carrot DH1. A total of 21 *TPS* genes were cloned and recombinant proteins functionally characterized. Several of these genes encode enzymes whose products are predominant components of the carrot DH1 root terpene profile.
3. To determine the change of terpene metabolism in 4 colored cultivars under elevated temperature conditions, we analyzed terpene profiles of these cultivars grown under three different temperature regimes. We found substantial differences in volatile composition between different carrot cultivars and growth conditions. Also, we have shown, that some monoterpenes (*e.g.* α-pinene, β-myrcene, and limonene), and some sesquiterpenes such as γ-cadinene, and δ-cadinene, and z-γ-bisabolene were increased under elevated growth temperatures. The accumulation of these volatile compounds during the high temperature of growth could contribute to the undesired taste of the carrot roots. In correlation with these results, changes of transcript levels of selected *TPS* genes were determined.
4. We attempted to generate transgenic RNAi plants to knock down the gene *DcTPS1* and *DcTPS2*, of which *DcTPS1* is responsible for the synthesis of the predominant carrot sesquiterpene compound (*E*)-β-caryophyllene. Unfortunately, no stable RNAi lines could be established for both of these genes due to possible toxic off-target effects.

Changes in direction from that in the original proposal

We followed the original proposal with no major changes.

Publications for Project IS-4745-14R

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Published	Other	*Yahyaa, Y., Tholl, D., Cormier, G., Jensen, R., Simon, P.W., Ibdah, M.*	Identification and characterization of terpene synthases potentially involved in the formation of volatile terpenes in carrot (Daucus carota L.) Roots	***J. Agric. Food Chem.***	63 : 4870-4878 2015	Joint
Published	Other	*Muchlinski, A., Ibdah, M., Ellison, S., Senalik, D., Simon, P.W., Tholl, D*	Identification and biochemical characterization of terpene synthases involved in carrot flavor and aroma and their response to elevated temperature.	***To be submitted to The Plant Journal***	: 2018	Joint
Published	Abstract - Poster	*Mosaab Yahyaa, Einat Bar, Neeraj Kumar Dubey, Dorothea Tholl, Philipp W. Simon, Efraim Lewinsohn, Mwafaq Ibdah*	Approaches to Understand the Complexity of Carrot (Daucus carota L.) Root Norisoprenoid Flavor.	***Gordon Research Conferences: Plant Volatiles Ventura CA, USA***	:	Joint
Published	Abstract - Poster	*Mossab Yahyaa, Dorothea Tholl, Guy Cormier, Roderick Jensen, Philipp W. Simon, Mwafaq Ibdah*	How Temperature Stress Changes Carrot Flavor: Elucidating the Genetic Determinants of Undesired Taste in Carrots	***Agriculture and Climate Change conference, Amsterdam, Netherlands***	: 2015	Joint
Published	Abstract - Poster	*Andrew Muchlinski, Shelby Ellison, Douglas Senalik, Mwafaq Ibdah, Philipp W. Simon, Dorothea Tholl*	Identification of the genetic determinants of volatile aroma in carrot (Daucus carota) roots,	***Terpnet 2017, 13th International meeting on biosynthesis, functions and synthetic biology of isoprenoids, Dalian University, China (2017).***	:	Joint
Published	Abstract - Poster	*Andrew Muchlinski, Shelby Ellison, Douglas Senalik, Mwafaq Ibdah, Philipp W. Simon, Dorothea Tholl*	Identification of the genetic determinants of volatile aroma in carrot (Daucus carota) roots	***Translational Plant Sciences Minisymposium, Virginia Tech (2017).***	:	Joint
Published	Abstract - Poster	*Andrew Muchlinski, Shelby Ellison, Douglas Senalik, Mwafaq Ibdah, Philipp W. Simon, Dorothea Tholl*	Identification of the genetic determinants of volatile aroma in carrot (Daucus carota) roots	***Gordon Research Conferences: Plant Volatiles Renaissance Tuscany Il Ciocco, USA (2018)***	:	Joint
Published	Abstract - Presentation	*Andrew Muchlinski, Shelby Ellison, Douglas Senalik, Mwafaq Ibdah, Philipp W. Simon, Dorothea Tholl*	Identification of the genetic determinants of volatile aroma in carrot (Daucus carota) roots	***Work-In-Progress Seminar Series, Virginia Tech (2018)***	:	Joint

Supplemental Table 1: Levels of mono and sesquiterpene and other volatile compounds, for which no authentic standards are available, and total content of mono and sesquiterpene and other volatile compounds in different carrot varieties at 11 weeks after sowing (16/12°C (±1°C), 22/18ºC (±1°C), and 28/24°C (±1°C) day/night). Peak areas for total mono and sesquiterpene and other content include all compounds from this table and Table 1.

	Relative volatiles composition (%)												
	16-12°C				**22-18°C**				**28-24°C**				
Compound	Orange	Red	Purple	Yellow	Orange	Red	Purple	Yellow	Orange	Red	Purple	Yellow	IC[a]
Tricyclene	n.d.	n.d.	n.d.	n.d.	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.01	0.04	0.05±0.00	0.04±0.01	0.03±0.00	RI, MS
α-Thujene	0.01±0.00	0.03±0.00	0.04±0.00	0.01±0.00	0.02±0.00	0.04±0.01	0.04±0.00	0.02±0.00	0.12±0.03	0.20±0.03	0.47±0.02	0.15±0.02	RI, MS
o-Cymene	0.09±0.01	0.73±0.02	0.06±0.04	0.30±0.00	0.14±0.01	2.07±0.10	0.15±0.01	0.6±0.01	0.30±0.05	2.80±0.32	1.56±0.40	4.42±0.20	RI, MS
β-Phellandrene	n.d.	1.55±0.16	n.d.	1.09±0.16	1.02±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
p-Menth-2,8-diene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.05±0.01	RI, MS
p-Cymenene	n.d.	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	0.12±0.04	0.06±0.01	0.13±0.01	0.42±0.05	RI, MS
1,3,8-*p*-Menthatriene	0.06±0.00	0.03±0.00	0.04±0.01	0.17±0.01	0.02±0.00	n.d	0.02±0.00	0.04±0.02	0.07±0.01	0.13±0.02	0.04±0.01	0.10±0.04	RI, MS
cis-*p*-Menth-2-en-1-ol	0.04±0.01	0.18±0.03	0.07±0.00	0.25±0.04	n.d	n.d	n.d	n.d	0.03±0.01	0.33±0.01	0.06±0.01	0.11±0.02	RI, MS
trans Limonene oxide	n.d.	n.d.	n.d.	0.09±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
p-Mentha-1,5,8-triene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.02±0.35	0.10±0.02	0.06±0.00	0.68±0.17	RI, MS
Neo-allo-ocimene	0.03±0.01	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	0.04±0.01	0.04±0.02	0.04±0.00	n.d	RI, MS
Epoxy Terpinolene	0.10±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
trans-*p*-Menth-2-en-1-ol	n.d.	0.13±0.01	0.05±0.00	0.38±0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11±0.03	RI, MS
1,8-Menthadien-4-ol	0.25±0.07	0.08±0.02	0.06±0.01	0.74±0.24	0.02±0.00	0.03±0.01	0.02±0.01	0.08±0.00	0.76±0.23	0.39±0.01	0.91±0.12	0.30±0.06	RI, MS
p-Cymen-8-ol	0.12±0.06	0.05±0.01	0.04±0.01	0.46±0.17	n.d	n.d	0.02±0.00	0.05±0.01	0.69±0.18	0.29±0.01	0.93±0.03	0.42±0.13	RI, MS
cis-Piperitol	0.01±0.00	0.05±0.01	0.03±0.00	0.04±0.01	n.d	n.d	n.d	n.d	n.d	0.03±0.00	0.04±0.00	0.02±0.00	RI, MS
trans-Piperitol	0.01±0.00	0.05±0.01	0.02±0.00	0.04±0.000	n.d	n.d	n.d	n.d	0.19±0.04	0.49±0.17	0.10±0.02	0.09±0.01	RI, MS
Fenchyl acetate	n.d.	n.d.	n.d.	n.d.	0.01±0.00	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
cis-Chrysanthenyl acetate	n.d.	0.03±0.00	n.d.	n.d.	n.d	0.01±0.00	n.d	n.d	n.d	0.36±0.05	n.d	n.d	RI, MS
trans-Pinocarvyl acetate	n.d.	n.d.	n.d.	n.d.	0.01±0.00	0.02±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Mertenyl acetate	n.d.	n.d.	n.d.	n.d.	0.01±0.00	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
trans-Carvyl acetate	0.01±0.00	n.d.	n.d.	0.01±0.00	0.04±0.00	n.d.	n.d.	0.04±0.00	n.d.	n.d.	n.d.	0.06±0.01	RI, MS
cis-Piperitol acetate	n.d.	0.29±0.05	0.06±0.00	0.13±0.01	n.d	0.01±0.00	n.d	n.d	0.01±0.00	0.02±0.00	n.d	n.d	RI, MS
Bicycloelemene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08±0.01	n.d.	n.d.	n.d.	RI, MS

δ-Elemene	n.d.	n.d.	0.03±0.00	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
cis-Carvyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03±0.01	n.d.	n.d.	n.d.	0.02±0.00	RI, MS
α-Longipinene	n.d.	0.01±0.00	0.01±0.00	n.d.	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.03±0.00	0.01±0.00	0.02±0.00	n.d	RI, MS
Cloven	n.d.	n.d.	n.d.	0.01±0.00	n.d	n.d	n.d	n.d	0.04±0.00	n.d	n.d	n.d	RI, MS
α-Ylangene	n.d.	0.01±0.00	0.02±0.00	n.d.	n.d	0.01±0.00	n.d	0.01±0.00	n.d	0.05±0.00	0.08±0.01	n.d	RI, MS
Cyclosativene	0.02±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Longipinene	n.d.	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06±0.02	n.d.	n.d.	0.09±0.01	RI, MS
α-Copaene	0.06±0.02	0.05±0.01	0.11±0.01	0.03±0.00	0.06±0.01	0.05±0.00	0.05±0.00	0.08±0.01	0.19±0.02	0.06±0.01	0.24±0.03	0.11±0.01	RI, MS
β-Cubebene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10±0.00	n.d.	n.d.	n.d.	RI, MS
7-epi-Sesquithujene	0.05±0.01	0.04±0.01	0.05±0.00	0.06±0.01	0.63±0.03	0.03±0.01	0.09±0.01	0.10±0.01	0.20±0.03	0.10±0.02	0.12±0.02	0.29±0.03	RI, MS
β-Elemene	0.17±0.03	0.04±0.01	0.05±0.00	0.06±0.01	n.d	0.04±0.01	0.07±0.01	0.11±0.03	0.79±0.10	0.05±0.01	0.16±0.00	0.21±0.01	RI, MS
Italicene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.13±0.01	n.d.	RI, MS
Sesquithujene	n.d.	n.d.	0.13±0.01	0.15±0.01	1.48±0.12	0.05±0.01	0.50±0.07	0.40±0.10	n.d	n.d	n.d	2.07±0.08	RI, MS
α-cis-Bergamotene	0.35±0.01	n.d.	0.32±0.02	0.30±0.03	0.42±0.02	n.d	0.97±0.08	0.46±0.12	1.84±0.21		0.62±0.09	3.36±0.44	RI, MS
2,5-Dimethoxy-p-cymene	n.d.	0.24±0.03	n.d.	n.d.	n.d.	0.17±0.01	n.d.	n.d.	n.d.	0.05±0.02	n.d.	n.d.	RI, MS
Aromadendrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11±0.02	n.d.	RI, MS
α-Barbatene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.33±0.13	n.d.	n.d.	RI, MS
α-trans-Bergamotene	2.14±0.48	1.10±0.04	0.98±0.06	1.00±0.09	2.97±0.13	1.32±0.09	2.20±0.08	1.66±0.14	0.12±0.02	0.82±0.30	0.22±0.04	0.61±0.05	RI, MS
Sesquisabinene	0.22±0.00	0.18±0.03	0.18±0.01	0.10±0.01	0.59±0.04	n.d.	0.40±0.04	0.25±0.01	n.d.	0.18±0.09	n.d.	n.d.	RI, MS
Acora-2,4(15)-diene	n.d.	0.06±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Isobazzanene	n.d.	n.d.	n.d.	n.d.	n.d.	0.10±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
α-Himachalene	n.d.	n.d.	n.d.	2.4±0.0	n.d.	n.d.	0.23±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
cis-Thujospene	n.d.	n.d.	n.d.	n.d.	0.30±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Allo-aromadendrene	0.12±0.00	n.d.	0.13±0.02	n.d.	n.d	n.d	n.d	0.13±0.01	0.09±0.01		0.09±0.01	0.11±0.01	RI, MS
β-Santalene	n.d.	n.d.	n.d.	0.04±0.00	0.13±0.00	n.d.	0.21±0.01	0.07±0.03	n.d.	n.d.	n.d.	n.d.	RI, MS
Epi-*β*-Santalene	0.06±0.00	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Barbatene	n.d.	n.d.	n.d.	n.d.	n.d.	0.17±0.06	n.d.	n.d.	n.d.	0.25±0.11	n.d.	n.d.	RI, MS
γ-Himachalene	n.d.	n.d.	n.d.	n.d.	0.06±0.01		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Dauca-5,8-diene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.00	0.18±0.02	0.04±0.01	n.d.	RI, MS
γ-Muurolene	n.d.	n.d.	0.05±0.01	0.05±0.01	0.07±0.00	0.90±0.13	n.d	0.06±0.01	n.d	0.07±0.03	0.15±0.03	n.d	RI, MS
Germacrene D	0.14±0.01	0.48±0.13	n.d.	n.d.	0.49±0.03				n.d.	1.07±0.12	0.04±0.01	0.01±0.00	RI, MS
β-Acoradiene	n.d.	2.59±0.69	n.d.	n.d.	n.d	0.09±0.01	n.d	n.d	n.d	2.45±0.21	n.d		RI, MS
γ-Curcumene	0.15±0.01	n.d.	0.32±0.07	0.32±0.05	0.44±0.04		0.30±0.04	0.23±0.04	0.03±0.01	1.35±0.04	0.82±0.02	0.10±0.01	RI, MS

α-Curcumene	n.d.	0.33±0.07	0.4±0.14	0.12±0.14	n.d.	0.38±0.05	0.57±0.07	0.22±0.02	n.d.	2.30±0.24	2.87±0.47	0.52±0.02	RI, MS
Amorpha-4,11-diene	n.d.	n.d.	n.d.	n.d.	n.d.	0.87±0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
δ-Amorphene	n.d.	0.04±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
γ-Patchulene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04±0.01	n.d.	RI, MS
γ-Amorphene	0.31±0.04	n.d.	0.19±0.08	0.24±0.04	0.61±0.04	n.d	n.d	0.28±0.02	n.d	n.d	0.17±0.04	n.d	RI, MS
cis β-Guaiene	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.00	n.d.	n.d.	n.d.	RI, MS
trans-Muurola-4(14),5-diene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03±0.00	n.d.	0.06±0.01	n.d.	n.d.	n.d.	RI, MS
β-Selinene	0.02±0.00	n.d.	n.d.	0.03±0.01	n.d	n.d	0.02±0.00	0.07±0.01	n.d	n.d	n.d	n.d	RI, MS
α-Bulnesene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07±0.03	n.d.	n.d.	RI, MS
α-Zingberene	0.06±0.00	0.27±0.06	0.28±0.07	0.56±0.05	n.d	0.46±0.10	0.41±0.12	1.10±0.10	n.d	0.31±0.07	0.80±0.13	0.01±0.00	RI, MS
Eremophilene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.00	n.d.	n.d.	n.d.	RI, MS
Bicyclogermacrene	n.d.	0.10±0.01	0.13±0.03	0.10±0.02	0.51±0.03	n.d	0.20±0.04	n.d	0.18±0.08	0.23±0.08	0.13±0.02	n.d	RI, MS
Viridiflorene	0.06±0.01	n.d.	0.02±0.00	n.d.	0.03±0.00	n.d	n.d	n.d	n.d	n.d	n.d	0.02±0.00	RI, MS
α-Muurolene	0.03±0.00	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	n.d	n.d	0.16±0.00	0.01±0.00	RI, MS
Z-α-Bisabolene	n.d.	0.44±0.07	n.d.	0.17±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Himachalene	0.03±0.00	n.d.	n.d.	0.06±0.00	0.21±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
γ-Himachalene	n.d.	n.d.	n.d.	n.d.	n.d.	0.34±0.06	n.d.	0.17±0.01	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Curcumene	0.31±0.06	n.d.	n.d.	n.d.	1.21±0.39	n.d.	n.d.	n.d.	0.04±0.01				RI, MS
Z-γ-Bisabolene	n.d.	1.17±0.29	0.58±0.05	10.76±0.47	4.44±0.40	15.77±1.94	1.45±0.20	9.49±0.67		5.48±0.71	1.98±0.46	1.11±0.03	RI, MS
γ-Cadinene	n.d.	n.d.	0.04±0.01	0.07±0.03	n.d	n.d	0.15±0.01	0.18±0.02	0.02±0.00	n.d	0.58±0.03	0.18±0.00	RI, MS
δ-Cadinene	0.02±0.00	0.05±0.04	0.07±0.01	0.14±0.03	0.06±0.00	0.10±0.03	0.06±0.03	0.13±0.04	0.04±0.00	0.2±0.03	1.17±0.19	0.05±0.00	RI, MS
Zonarene	0.02±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Myristicin	n.d.	n.d.	n.d.	n.d.	n.d.	0.28±0.02	0.40±0.04	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Sesquiphellandrene	0.29±0.03	n.d.	n.d.	n.d.	0.62±0.02	n.d	n.d	n.d	0.03±0.00	n.d	n.d	0.08±0.01	RI, MS
E-γ-Bisabolene	0.05±0.01	20.17±4.05	21.48±1.34	16.40±5.09	0.65±0.09	6.25±0.47	24.03±1.46	17.11±3.67	0.01±0.00	0.40±0.02	6.54±0.37	1.10±0.10	RI, MS
γ-Cuprenene	n.d.	n.d.	n.d.	0.26±0.01	0.06±0.02	n.d	n.d	n.d	n.d	n.d	n.d	n.d	RI, MS
Kessane	0.03±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	RI, MS
E-α-Bisabolene	n.d.	n.d.	0.13±0.06	0.11±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	RI, MS
α-Cadinene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.00	0.57±0.11	0.03±0.01	0.01±0.00	RI, MS
Trans cadina-1,4-diene	n.d.	n.d.	n.d.	n.d.	0.14±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
cis-Sesquisabinene hydrate	0.01±0.00	n.d.	n.d.	n.d.	0.07±0.02	n.d	n.d	n.d	n.d	n.d	n.d	n.d	RI, MS
Caryophyllenyl alcohol	0.01±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Trans sesquisabinene hydrate	n.d.	n.d.	n.d.	n.d.	0.09±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Geranyl isovalerate	n.d.	n.d.	n.d.	n.d.	0.45±0.03	n.d.	0.42±0.10	n.d.	0.78±0.13	0.08±0.02	1.83±0.08	0.12±0.03	RI, MS

Humulene epoxide 2	n.d.	n.d.	n.d.	0.06±0.02	n.d.	n.d.	n.d.	n.d.			0.11±0.01	n.d.	RI, MS
Carotol	n.d.	n.d.	n.d.	n.d.	0.06±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Cis calamenen-10-ol	n.d.	n.d.	n.d.	n.d.	n.d.	0.05±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Gossonorol	n.d.	0.04±0.01	0.05±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06±0.01	n.d.	n.d.	RI, MS
Allo Aromadendrene epoxide	0.07±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
β-Eudesmol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.06±0.03	n.d.	n.d.	n.d.	RI, MS
α-Caryophylladienol	n.d.	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	0.29±0.06	RI, MS
Z-*α*-Santalol	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Caryophylla-3,8(13)-dien-5-*β*-ol	0.05±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21±0.10	n.d.	n.d.	0.03±0.01	RI, MS
Vulgarol B	n.d.	n.d.	n.d.	0.16±0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Allohimachalol	0.03±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
2,6-dimethyl cyclohexanol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.01	n.d.	n.d.	RI, MS
Dodecene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.00	RI, MS
Dodecane	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.00	n.d.	n.d.	0.10±0.04	0.05±0.02	0.05±0.01	0.07±0.01	RI, MS
Theaspirane B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.01±0.00	n.d.	n.d.	n.d.	0.07±0.02	n.d.	RI, MS
Daucene	n.d.	0.77±0.13	n.d.	n.d.	1.05±0.08	1.25±0.16	0.02±0.00	n.d	n.d	2.64±0.41	0.07±0.02	n.d	RI, MS
Isodaucene	n.d.	0.16±0.02	n.d.	n.d.	n.d.	n.d.	0.07±0.01	n.d	0.01±0.00	n.d	n.d	n.d	RI, MS
Elemicin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Tetradecanal	0.21±0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
Isopropyl tetradecanoate	n.d.	n.d.	n.d.	n.d.	n.d.	0.02±0.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS
6-methoxy oxymellein	n.d.	n.d.	n.d.	n.d.	n.d.	0.29±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	RI, MS

IC[a]: identification criteria. The identification criteria based on mass spectra matching with the standard NIST-14.L and Wiley 10N.I library (MS), comparison of retention index (RI). bn.d: not detected. The results shown are an average of three biological replicates.